Comparison of the bacteriolytic activity and the pattern of bacteriolytic compounds in the saliva, stomach and small intestine of the haematophagous bug *Triatoma infestans* (Reduviidae) – a review

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Zusammenfassung: Bei den mehr als 150 Arten der Unterfamilie Triatominae (Reduviidae, Hemiptera) saugen alle postembryonalen Stadien Blut. In Lateinamerika übertragen sie *Trypanosoma cruzi* (Trypanosomatidae, Kinetoplastida), den Erreger der Chagas Krankheit, der sich im Darmlumen der Vektoren entwickelt. In den Speicheldrüsen und im Darm finden sich viele Bakterien, die z. T. eine symbiontische Funktion haben. Ihre Entwicklung wird durch antibakterielle Faktoren beeinflusst. Beim Speichel ungefütterter Nymphen der Triatomine *Triatoma infestans* sowie der Nymphen bis zu 15 Tage nach der Fütterung fand sich bei der spectrophotometrischen Untersuchung der bakteriolytischen Komponenten bei pH 3 – pH 10 eine stärkere Lyse lyophilisierter *Micrococcus luteus* v.a. bei pH 4 und pH 6. Nach der Fütterung änderte sich der Level der Lyse bei pH 4 kaum, verdoppelte sich aber vom 3. auf den 5. Tag bei pH 6. Bei der SDS-Gelelektrophorese mit einer Zymographie und der Inkubation bei pH 4 bzw. pH 6, z. T. gefolgt von Inkubationen in Wasser, fanden sich bei pH 4 acht Banden zwischen 14,1 und 38,5 kDa mit einer Lyse von *M. luteus*, wobei die stärkste Aktivität bei 24,5 kDa vorlag. Die Inkubation bei pH 6 führte nur bei 15,6, 17,1 und 31,4 kDa zur Lyse. Nach der Fütterung nahm die Lyse bei 17 kDa stark zu. Bei der Spektrophotometrie von Extrakten des Magens und Dünndarms ungefütterter Nymphen und bis zu 50 Tagen nach der Fütterung wurde *M. luteus* am stärksten bei pH 4 und pH 7 lysiert. Der Symbiont *Rhodococcus triatomae* wurde von allen Extrakten nicht lysiert. Nach der Fütterung war die Aktivität geringer, nahm dann zu und erst 50 Tage nach der Fütterung bei den 20 Tage hungernden Imagines drastisch ab. Die Aktivität der Magen-Extrakte war immer stärker als die der Extrakte des Dünndarms. In der Zymographie korrelierte die stärkere Aktivität mit Proteinen von 16 kDa. Insgesamt lagen sieben Lysebanden zwischen 15 und 40 kDa vor, mehr beim Dünndarm als beim Magen. Da bei Triatominen antibakterielle Proteine nur bis 22 kDa bekannt sind, werden die Lysebanden bei höheren molekularen Massen eventuell durch unbekannte Komplexe von antibakteriellen Proteinen und Verdauungsenzymen hervorgerufen.

Key words: bacteriolytic activity, bacteriolytic compounds, saliva, intestinal contenets, Triatominae

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Introduction

In Latin America, bloodsucking triatomines are vectors of the causative agent of Chagas disease, *Trypanosoma cruzi*. The intestinal tract is also colonised by many bacteria and fungi (reviewed by Vallejo & al. 2009), transmitted via coprophagy (Schaub 2020). Also the salivary glands of different species of triatomines possess a variety of bacteria (Lima & al. 2018). In the intestinal tract the mutualistic symbionts strongly develop in cardia and stomach, two regions of the anterior midgut. Within 7 days after feeding nymphs of the triatomines *Rhodnius prolixus* and *Triatoma infestans*, the numbers of symbionts increase about 30- and 80-fold in cardia and stomach, respectively. However, after the passage from the stomach to the small intestine, these concentrations are considerably reduced (Eichler & Schaub 2002). Those surviving and passed to the rectum are relevant for the coprophagic infection. Both, the strong development and the reduction, are surprising since the expression level of genes of antibacterial compounds in the stomach is much higher than in the small intestine (reviewed by Schaub 2021). Therefore, this

activity seems to act against nonsymbiotic bacteria. The saliva and the intestinal contents should contain antibacterial compounds to inhibit or regulate the microbiota. Several genes encoding antimicrobial peptides of different masses are expressed: defensins (4 kDa), pacifastin-like protease inhibitors (4 kDa), short-trialysins (6.1 kDa), diptericin (9 kDa), prolixicin (11 kDa), a single domain (7 kDa) and a double domain (11.5 kDa) of Kazal-inhibitors, TiAP (12.5 kDa), histones (13, 15 kDa), triatox (14.8 kDa), lysozymes (15 kDa), hemolysin-like proteins (16 kDa), attacins (20 kDa) and trialysins (22 kDa) (reviewed by Meiser & al. 2023). Since the importance of these peptides is unknown, we investigated the activity and the pattern of bacterial compounds.

Saliva was collected using unfed fifth instars and nymphs up to 15 days after feeding by holding the nymphs with bare hands by the first pair of legs and gently expiring air onto the head. The released saliva was visible at the tip of the proboscis. Stomachs and small intestines were dissected up to 50 days after feeding, homogenized and centrifuged at 16 000 × *g* for 15 min at 4 °C to use the resultant supernatants. At 30, 40 and 50 days after feeding, the nymphs had moulted to the adult stage. The antibacterial activity of the saliva and of extracts of stomach and small intestine of *T. infestans* was determined photometrically between pH 3 and pH 10. After electrophoretic separation, zymography under nonreducing conditions enabled the characterization of compounds lysing the Gram-positive bacterium *Micrococcus luteus* (Meiser & al. 2022, 2023).

Bacteriolytic activity and the pattern of bacteriolytic compounds in the saliva

Considering the pH-dependency of the activity, bacteriolytic activity against lyophilised *M. luteus* was stronger at pH 4 and pH 6 (Meiser & al. 2023). At pH 4, the activity was similar up to 15 days after feeding. At pH 6, this occurred only up to 3 days after feeding; it more than doubled between 3 and 7 days after feeding, followed by a constant level up to 15 days after feeding. In zymography under nonreducing conditions and after incubation at pH 4 and a staining in methylene blue, *M. luteus* was lysed at 15.6, 16.4, 17.1 and 24.5 kDa. After prolonged incubation in water, additional bands appeared at 14.1, 14.7, 21.4 and 38.5 kDa, but the strongest lysis occurred at 24.5 kDa. An incubation at pH 6 resulted in lysis regions at 15.6, 17.1 and 31.4 kDa. Comparing the zymographs of the saliva of unfed nymphs and of nymphs used 7 days after feeding, the activity at 17 kDa was stronger in fed nymphs (Meiser & al. 2023).

Bacteriolytic activity and the pattern of bacteriolytic compounds in the stomach and small intestine

Using the extracts of stomach and small intestine, the activity against *M. luteus* was the strongest at pH 4 and pH 7 and higher in the stomach than in the small intestine (Meiser & al. 2022). Symbiotic *Rhodococcus triatomae* were not lysed. Lysis of Gram-negative *Escherichia coli* showed a maximum at pH 7 in the stomach and at pH 5 in the small intestine. Comparing the time course at pH 4 and pH 7, the newly ingested blood reduced bacteriolytic activity of the stomach extracts against *M. luteus* by about 50 % at both pH values. It then increased, and in long starving adults at 50 days after feeding it was strongly reduced. In zymographs, the activity against *M. luteus* was mainly correlated to compounds of about 16 kDa. At different periods of time after feeding, seven bands of lysis showed up at 15, 16, 16.5, 19, 22, 36 and 40 kDa. More bands appeared using extracts of the small intestine than those of the stomach (Meiser & al. 2022).

Aspects of the different pH optima and the bacteriolytic compounds

Considering the pH-dependency, the saliva and the extracts of the two regions of the intestine possess higher activities at pH 4 and at pH 6 / pH 7. The higher pH value is optimal for the saliva with a pH of 6 – 7 (Yuda & al., 1997, Soares & al., 2006). The lower pH value might play a relevant role in the intestinal tract. In the stomach and small intestine strong pH variations occur. After the ingestion of blood with pH 7.4 acidification begins, reducing the pH of the contents in both stomach and small intestine to pH 5.2 (Balczun & al., 2012). Considering an acidification gradient from the wall of the intestine to the lumen, even lower pH values near the wall might occur (Meiser & al. 2022).

Focussing on the bacteriolytic compounds in zymography, the saliva induced nine bands of lysis, six in the range of 14.1 – 21.4 kDa and additional ones at 24.5, 31.4 and 38.5. In the intestinal contents seven bands of lysis occurred, very strong bands at about 16 kDa, but also at 19, 22, 36 and 40 kDa. In the

transcriptome analyses, only compounds up to 22 kDa had been identified (reviewed by Meiser & al. 2023). The bands at higher molecular masses presumably result from complexes of antimicrobial compounds and proteases. In the stable fly *Stomoxys calcitrans*, SDS-stable complexes of defensins and a serine protease are released into the intestine (Hamilton & al. 2002), and the saliva of triatomines contains sialocomplexes with histones, suggested to possess an antimicrobial activity (Santiago & al. 2020)

Conclusions

This review clearly shows the relevance of bacteriolytic compounds in the saliva, stomach and small intestine of *T. infestans* and highlights the necessity to identify the compounds of higher molecular masses. The higher level of activity in the stomach compared to the small intestine and the lysis resistance of the mutualistic symbiont indicate the task of the compounds: the regulation of the development of different bacteria in the intestinal tract. This is supported by *T. cruzi*, since an infection induces immune reactions to suppress the development of bacteria (reviewed by Schaub 2021). Factors responsible for the strong development of the mutualistic symbionts in the stomach and the reduction in the small intestine remain unknown. Presumably complexes of bacteriolytic compounds and digestive enzymes become evident.

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